OBSERVATIONS ON THE STRUCTURE OF DOUBLE MONSTERS IN THE EARTHWORM.[1]

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I. Introduction.

The work which has thus far been done on double monsters in the terricolous group of annelids is quite limited, since in only a few species, *Lumbricus trapezoides*, *Lumbricus terrestris*, *Allolobophora fœtida* and *Allolobophora trapezoides*, has this condition been observed. The first of these forms is described by Kleinenberg, the remaining three by Vedjovsky. These two investigators agree neither as to the method of union nor as to the cause of this phenomenon. It is not the object of this paper to give the factors influencing such monster formations, but merely to describe the manner in which the members are joined.

Material and Technique.—The material studied consists of twelve double embryos, the shortest member of which is at least 60 segments in length, the longest 125, one monster in which one individual consists of 16 the other 17 segments, 19 double gastrulæ and two embryos showing a bud on one side of the blastopore, and one case of an egg consisting of two distinct hemispheres connected by a band of large cells. The entire supply was obtained from Professor Patterson, of this University.

The fixing fluids employed were Meves, Gilson, and Bouin, all of which gave only fairly good results. Ehrlich's hæmatoxylin was used as a stain.

The species studied is *Helodrilus caliginosus trapezoides*, according to Professor F. Smith's identification.

Review of the Literature.—Before describing the individuals studied, it will perhaps be well to give a brief review of the results of the two authors above mentioned.

Kleinenberg found that the number of eggs in the capsules of *L. trapezoides* varies from three to eight of which usually one,

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though sometimes two or three, produces an embryo. The remaining ones, not becoming fertilized, disintegrate completely.

By a series of somewhat irregular cleavages the egg develops into a germinal bladder, one layer in thickness (Pl. IX., Fig. 2, Kleinenberg). Thereupon a two-layered condition begins to appear, one side of the cell mass becoming differentiated in advance of the other. At this pole all the cells divide rapidly except two, which become pushed in and covered over by the small blastomeres. From these two cells (mesoblasts) are derived the first rudiments of entoderm and mesoderm (Pl. IX., Fig. 4).

While this elongation is taking place a transverse furrow appears midway between the two ends extending almost entirely around, leaving the two hemispheres connected by only a few enlarged ectodermal cells.

When this stage has been reached the cells of the other pole begin to undergo the same changes and finally we have formed from each half an embryo joined in varying degrees to the other by a band of ectodermal cells. The separation is eventually effected by a series of rotations which usually result in the breaking of the uniting cord. When for one reason or another this is not accomplished we have true monstrosities in all degrees of coalescence.

According to Kleinenberg the union never extends to the internal organs but is confined to the external epithelium of the body wall.

The monsters formed in this manner are of the same or nearly the same size. There is however still another type of abnormality (Pl. IX., Fig. 10), *e. g.*, those showing bud formation. Kleinenberg explains their production as being due to a very unequal development of the two halves of the above mentioned cell mass.

In all his work he has found only a few cases in which two individuals did not emerge from one capsule and in those few exceptions rudiments of a second were usually found. For this reason he concludes that each egg produces two individuals normally.

Vedjovsky on the other hand holds this condition to be ab-

normal. He explains the formation of double embryos as being due to what he terms *Doppelfurchung*. The egg elongates in the direction of the animal pole and subsequently divides into two blastomeres either of equal or unequal size. They are designated as A and a. From each of these cells there is produced a quartet, one lying upon the other giving the appearance of micro- and macromeres in the cleavage of *Crepidula* or the eight cell stage of *Synapta*, depending on the proportionate sizes of A and a. Each of the quartets finally gives rise to an individual which remains attached to its companion. If the a cell is smaller than A, there is produced one well developed individual and a bud.

This author has found examples of monster formation in *Lumbricus terrestris*, *Allolobophora fœtida* and in *Allolobophora trapezoides*. In the first group he found two cases in both of which the two individuals were joined on the dorsal side through one segment. Only one monster was found among several hundred embryos of the second group. In the third group a large number was found which the author describes under three divisions.

A. Those monsters in which the individuals are united on the ventral side along the entire length of the body (Pl. 19, Fig. 14).

B. The cases in which the members are joined on the dorsal side (Plate 21, Fig. 7).

C. The double monsters in which the individuals are fused end to end. (*a*) Those in which both members are of similar length (Pl. 21, Fig. 9). (*b*) Those cases in which one member is rudimentary (Pl. 19, Fig. 12).

II. Observations.

Even though these two workers differ so markedly in their conclusions it will be noted from the following that the observations made by the writer are similar in many respects to those made by both of these men.

In this study of the monsters of *Helodrilus caliginosus trapezoides* it has been found that the greatest variety exists in the structure of the nervous system. There is in every case a complete union of the five layers of the body wall, and the

digestive tract is either fused or not fused, but the nervous system is modified in a variety of ways. The manner and extent of union of these individuals and their organ systems are best described in the following groups.

1. Those double monsters in which the union is dorsal. This group may again be subdivided into—

(*a*) Those in which the union extends to the alimentary tracts. Of these there are two examples, Nos. 95 and 171, Plate I. From Figs. 1 and 2, Plate I., it can be seen that the fusion in No. 171 is through one segment only, while in No. 95 it extends through five. A description of sections through this monster will serve to show the general relationship of the organ systems in the members of this group. Fig. 3, Plate I., is a section through the anterior portion showing the condition of the nervous system. It will be seen that there is almost a continuous band of nerve tissue extending around the pharynx. A study of the neighboring sections shows that this band is really complete. It will also show that the portion marked (*b*) in the figure enlarges into a bilobed brain and that a similar structure exists on the opposite side of the pharynx. At the same time there is also to be found at each end of the greatly elongated pharynx another set of ganglia. It would be impossible here to determine which are brain and which are ventral cord ganglia were it not for the presence of setæ (*s*). This at once leads to the conclusion that the ganglia at the end of the pharynx must be those of the ventral cord. This inference is further proven by a study of sections through a more posterior region, as shown in Fig. 4, Plate I. Here are no longer to be found the ganglia on either side of the pharynx but only those at the opposite ends. This section too shows the setæ in normal relationship with the ventral cord and the nephridæ in their natural position. They are shown only on one side but those of the other half can be seen in the next section.

It will be noted that the single pharynx is greatly elongated from end to end of the monster. Throughout the fused part it remains as one cavity dividing into two parts only when the point of separation of the two members is reached, one passing into each individual. Surrounding the pharyngeal cavity there

is a great mass of muscular tissue attached by thin strands to the body wall. This condition is exactly similar to that found in single individuals.

The sub-intestinal blood vessels are clearly visible (*v.b.v.*) in their normal position between the alimentary tract and the nerve cord. On each side of the muscular pharynx there lies a more or less regular vessel (*D.B.V.*) which when traced to the point of separation of the two members will be found to approach the center and then one passes into one individual, the other into the other, the vessel on the left side passing into the upper half, the one on the right into the lower half.

Though the other monster is formed by a coalescence of the two members through one segment only, that union is complete in every respect from body wall to pharynx. Fig. 5 is a longitudinal section through No. 171 showing a portion of one of the brain ganglia together with a small part of the nerve cord of each of the individuals. A study of the successive sections will show that the pharynx arises from a flattened portion which is lined with the same kind of epithelial cells as the rest of the digestive tract and which lies between the two members as indicated in Fig. 2, Plate I. (*m*). The ventral cords are found to be connected to the cerebral ganglia, of which there are two sets, one on each side of the mouth opening, by means of commissures as in No. 95.

The setæ, not shown in this figure, and the nephridæ lie along the same side of the body as the nerve cord.

(*b*) Those in which the alimentary tracts have not become united. Nos. 91, 92, 142 and 173 shown in Plate II as Figs. 6, 7, 8 and 9 illustrate this group. It will be noted that though only one definite pair of segments appear to be fused in the first three cases, there is in each instance an irregular mass of tissue between the separated edges of the fused pair of segments. Whether these masses are modified segments has not been determined.

In all four instances the nerve cords are formed on the side opposite the line of fusion. These cords are joined to the bilobed brains in a manner very similar to that found in No. 95 (Fig. 3). It is to be noted that the union of these individuals

is the same in kind and extent as in the group already described except for the digestive tract. In every one of the four there is distinguishable a separate alimentary tract for each member beginning with the mouths. These structures lie very near the nerve cord and are bounded on the inner side by a mass of muscles similar to those which usually lie on either side of the elongated fused pharynx.

The fixation of the blood vessels was such that they could not be studied.

The members of these groups to which belong half the specimens studied, have undoubtedly been fused along the dorsal side. The relative positions of setæ, nephridæ, and nerve cord are positive proof thereof. Vedjovsky gives a number of figures, very similar to Figs. 3 and 4, in his paper but he explains the union as being along the ventral side. It is impossible to disprove this conclusion for the two members were joined along their entire lengths. In the cases described in this paper, the union was never through more than five segments If, then, the coalescence had been other than dorsal, it is very probable that the nerve cord together with the setæ and excretory organs would have swung around to their natural position in the separated portions of the two individuals. This, however, is not the case. Therefore, the conclusion must be drawn that at least in these instances the union is no other than dorsal.

It is very probable that the one case described by Vedjovsky is also one of dorsal instead of ventral union, and that the cerebral ganglia are composed of half from each member instead of this being the case for the ventral cords, as that author supposes. If those individuals in which the digestive tract was separate had not been found it would not be possible to make this statement for it might be argued that each side of the pharynx was formed from the alimentary tract of one member. But since those cases have been found it can be easily seen that a joining of these structures would have resulted in forming a greatly elongated organ similar to that shown in Vedjovsky's figures. Furthermore, if the union in these forms had been ventral, the pharynges would certainly not have been found in the positions in which they lie, but would have been much nearer together in the central portion and side by side instead of end to end.

2. Those cases in which the union is latero-dorsal. Thus far only one example has been found of this group, individual No. 70 shown in Fig. 10, Plate II. Figs. 11 and 12, Plate II., show sections through the joined region which in this case extends through three segments. The first is very much more anterior than the second as is indicated by the presence of the cerebral ganglia (*bg*) of which there is in this monster only one set. The commissures extending from it can be seen in the same figure in which is also present the beginning of one of the nerve cords (*nc*). A study of the next few sections shows the connection of the brain to the two cords and also a connecting band between these two structures. In this manner the single greatly elongated alimentary tract, which in this case is the result of a union, is completely surrounded by nerve tissue. Fig. 12 gives a section through a more posterior region showing the relative positions of the ventral cords.

In no part of the joined portion can there be found less than eight pairs of setæ indicating that the union does not extend beyond the sides of the individuals.

The nephridæ also are in their normal number and relationship to the ventral cord.

3. This is the group in which the union is end to end and in which the cerebral ganglia are to be found on the opposite side of the digestive tract from the ventral cord. It can be seen from an inspection of Figs. 13, 14 and 15, external drawings of Nos. 90, 93 and 2, that the extent of union is limited to an unusually small area. Sections show that the union is across the dorsal side, for the ventral cords lie on the sides opposite that area, while the cerebral ganglia lie on the same side.

The digestive tracts have a common origin in each of the three monsters arising from a single mouth opening from which the pharynx passes into each individual. There is no noticeable difference in the structure of any of the fused organs of these forms as compared with those of No. 95 except in the nervous system.

A study of No. 90 will show a condition of that system somewhat different from any so far described. The ventral cord of each individual lies on the side opposite the union while the

brains lie on the same side and are connected to the cords by means of commissures, a condition very similar to that found in normal single individuals. The brains however in this instance are joined to each other. In one of the individuals the commissural connection between brain and cord can be seen beyond the point of separation, but in the other member the two ganglia of the brain seem to be separated and joined separately to the cerebral ganglia of the first member.

In No. 93, shown in Fig. 14, there is present only one set of cerebral ganglia greatly elongated. It lies on that side of the pharynx opposite the cords and is joined to that structure in one member by two commissures, to that in the other by only one.

A study of sections through No. 2 reveals a condition similar to that in No. 90. There are two distinct sets of cerebral ganglia, one joined to each nerve cord as in normal individuals and a connection between the two brains much as between the two cords in No. 70.

4. That group of double monsters in which the union between the two individuals has been side to side with both mouth openings on the same side, illustrated by Nos. 67 and 1.

In No. 1 the union is through one segment only as indicated in Fig. 16, Plate III. There is a single mouth opening lined with the columnar epithelial cells, from which the digestive tract of each individual passes inward. There can be seen on one side of the pharynx in a transverse section a single set of cerebral ganglia which is joined to the ventral cords by commissural strands extending over the pharynx. There are no such structures to be found on the under side of the alimentary tract. On the opposite side of the section there can be traced a connection between the cords themselves. Fig. 17, Plate III., is a section through the united portion of No. 1 showing the single pharynx, cerebral and ventral ganglia and a portion of the strand of nerve tissue connecting the two cords.

A study of sections through No. 67, shown in Fig. 18, Plate III., will show that the two mouth openings have been joined into one, and that the ventral cords lie on one side of the common pharynx and the cerebral ganglia on the other. There is only one set of brain ganglia to be found in this monster, but,

unlike all other cases, there are three distinct commissural strands joining it to the nerve cords, one extending over the top of the pharynx, the other two below the point of union of the two digestive tracts forming a triangle with each other. Fig. 19, Plate III., is a cross section of these last mentioned strands and their connection to the two cords. As in No. 70 there is in this monster also a limited connection between the two cords.

5. Cases in which the two individuals are extremely unequal in size, *e. g.*, bud formation. Thus far there have been found two such cases, a section of one being shown in Fig. 20, Plate III.

III. Discussion.

It will be remembered that reference was made to the difference in view concerning the origin of double monsters in an earlier part of this paper. Vedjovsky maintains that the formation of two embryos from one egg is abnormal, whereas Kleinenberg maintains the opposite. A difference of this kind may be explained on the basis of the difference in the forms worked on by these two investigators. This paper is a study of a form very similar, in its mode of development, to that described by Kleinenberg. It has been found that it is quite common, not only for monsters to appear in this group, but also for two individuals to emerge from one capsule. Out of the 184 cocoons opened 57 contained only one individual, 101 two, and 25 eggs in various cleavage stages and 1 four embryos. Thirty-five of the 101 cases were in the form of monsters. Six cases have been noted in which two or more eggs were present but in four of these only one was undergoing development while the others were in various stages of degeneration. One egg was found which had just reached a stage in development sufficiently far advanced to show the division into two hemispheres and the connecting band of larger cells very similar to Kleinenberg's Fig. 6. Three others were found which had begun to elongate in a manner very similar to the above.

It would certainly be safe to conclude that it is a common occurrence for one egg of this species to give rise to two individuals. If this were not the case it would seem rather unusual to find so large a number of cases in which two and only two

individuals emerge from one capsule when the number of eggs found in one capsule varies ordinarily from three to eight. The elongation of those eggs sufficiently far advanced in development to show this phenomenon is still further indication of this same thing.

Kleinenberg attributes the cause of this double embryo formation to the entrance of two sperm into a single egg setting up two points of activity. In the light of more modern discoveries this explanation no longer holds good. Vedjovsky suggests the possibility of temperature and moisture changes, and exposure to air bearing their influence on the egg and causing its abnormal development. Until further studies are made along this line it will be impossible to state any definite causes of monster formation.

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Explanation of Plate I.

Helodrilus caliginosus trapezoides.

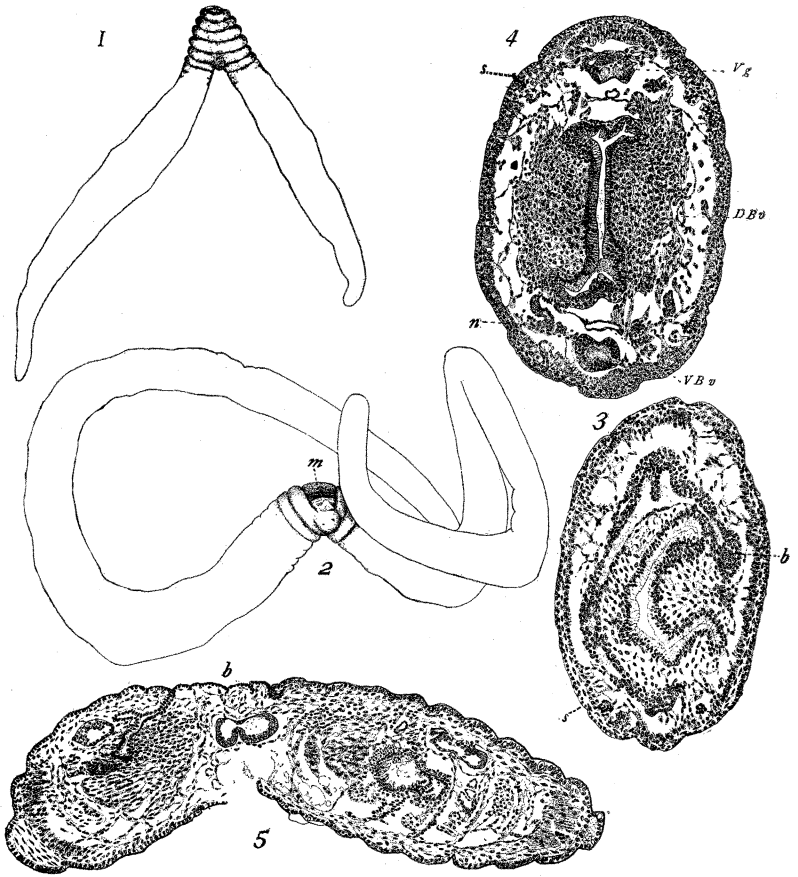
1. No. 95, showing union through five segments.

2. No. 171, showing union through one segment.

3. Section through the anterior portion of No. 95, showing the pharynx fused and the extension of the nerve tissue almost entirely around the pharynx. *b*, cerebral ganglia; *s*, setæ.

4. A section through a more posterior region of the same monster. *Vg*, ventral ganglia; *DBv*, dorsal blood vessel; *VBv*, ventral blood vessel; *n*, nephridium; *s*, seta.

5. A longitudinal section through No. 171, showing a portion of the cerebral ganglia *b*, and also parts of the ventral cords.



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Explanation of Plate II.

Helodrilus caliginosus trapezoides.

6. No. 91.
7. No. 92.
8. No. 172.

} Fused through one segment only with irregular masses between the separate edges of the united segment.

9. No. 173, united through four segments.

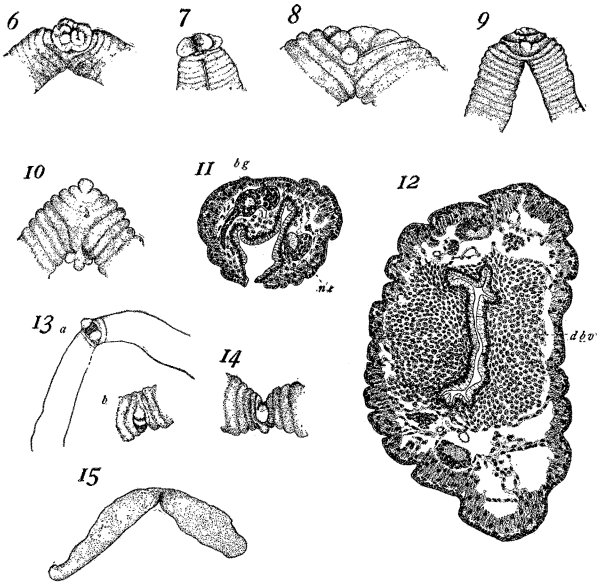
10. No. 70, united through four segments.

11. A section through the anterior portion of No. 70, showing a single set of cerebral ganglia (*bg*) and one of the ventral ganglia (*nc*).

12. A more posterior section of the same monster showing two ventral ganglia.

13*a* and *b*. No. 90.
14. No. 93.
15. No. 2.

} All show very limited connection.



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Explanation of Plate III.

Helodrilus caliginosus trapezoides.

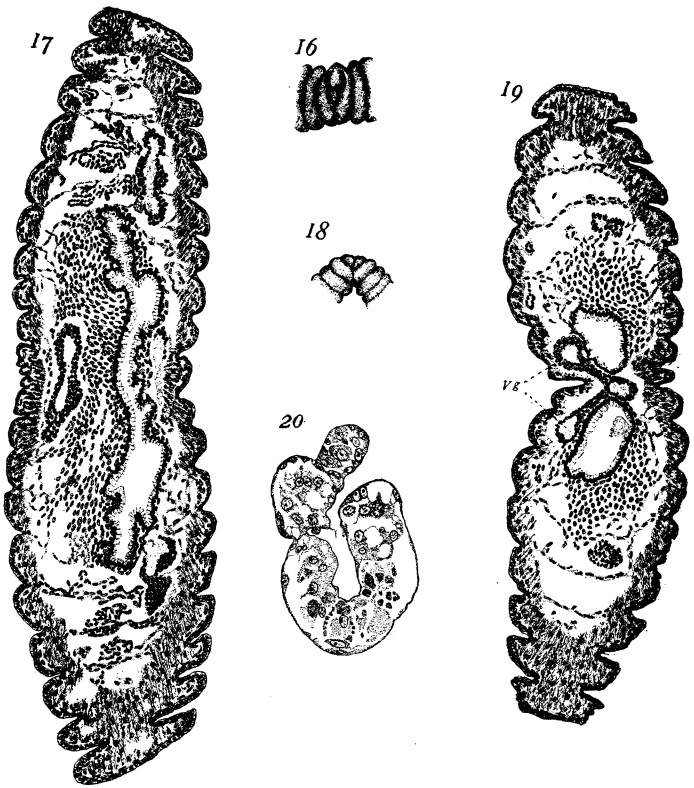
16. No. 1 viewed from the side of the mouth opening.

17. A section through No. 1 horizontal to the mouth opening, showing the cerebral ganglia on one side of the pharynx and the cord tissue on the opposite side.

18. No. 67, united through two segments.

19. A transverse section through the posterior part of the united portion showing the cerebral ganglia joined to the ventral ganglia (*vg*) by commissures.

20. A section through a gastrula stage showing bud formation.



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